Development of a molecular subtyping method for *Salmonella* Typhimurium combining different types of markers in a Luminex xTAG assay

V. Wuyts[1,2], S. Bertrand[3], N. Roosens[2], K. Marchal[1,4] and S. De Keersmaecker[2]

[1]Department of Microbial and Molecular Systems, KU Leuven, Leuven, Belgium; [2]Platform Biotechnology and Molecular Biology, Scientific Institute of Public Health, Brussels, Belgium; [3]Bacterial Diseases Division, Communicable and Infectious Diseases, Scientific Institute of Public Health, Brussels, Belgium; [4]Department of Plant Biotechnology and Bioinformatics, Ghent University (VIB), Ghent, Belgium.

veronique.wuyts@wiv-isp.be

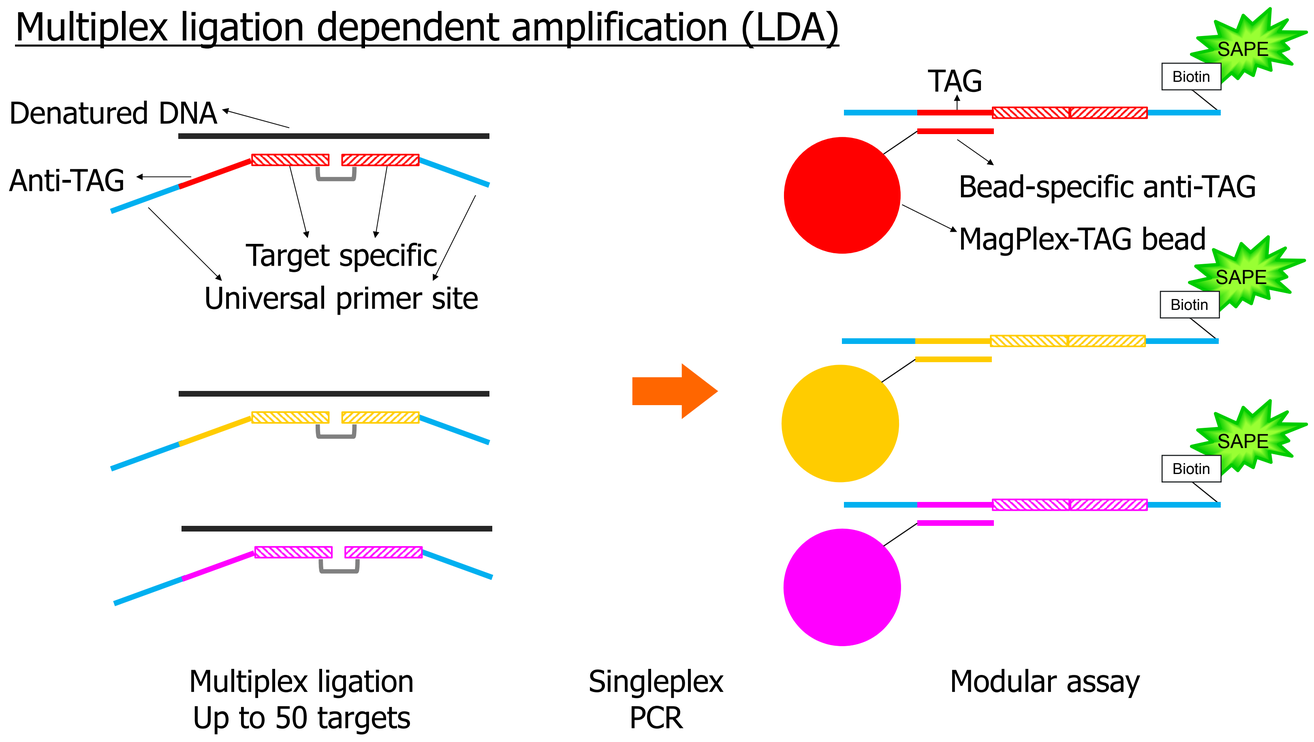
CONTEXT

Surveillance, and identification, tracking and confinement of outbreaks of *Salmonella* Typhimurium (STM):
need for inexpensive, rapid, sufficiently discriminative and robust method for subtyping
⇒ Molecular method: different types of markers available
⇔ Constraint: level of multiplex feasible ⇒ Luminex technology

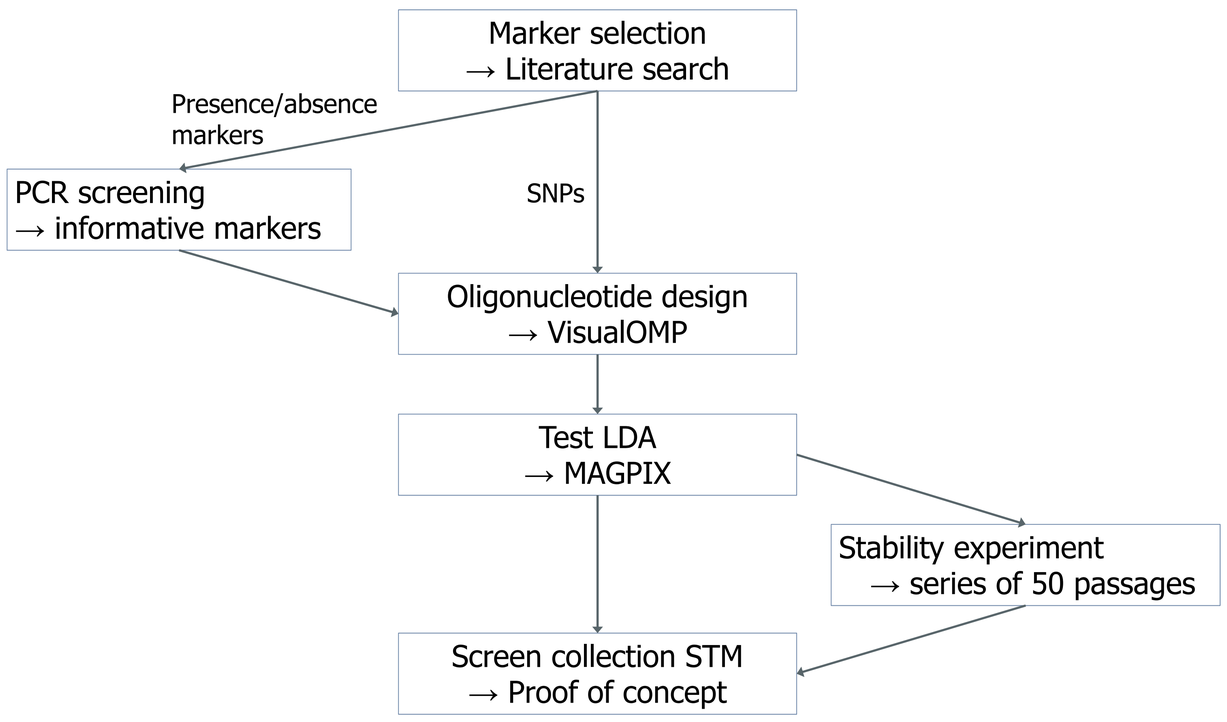
LUMINEX ASSAYS FOR NUCLEOTIDES

Feature	Direct hybridization	LDA	TSPE
Usage	SNPs, multiple polymorphisms, unrelated sequences		
Beads	MagPlex	MagPlex-TAG	MagPlex-TAG
Required oligos	Labelled multiplex primer pairs and modified capture probes	Modified multiplex probe pairs and labelled universal primer pair	Multiplex primer pairs and modified probe
Coupling of oligo to beads	Yes	No	No
Multiplex	PCR	Ligation	PCR
Addition of markers	Redesign mPCR	No redesign	Redesign mPCR
Hybridization of target oligo to beads	Optimize: 45-55° C	Standard: 37° C	Standard: 37° C
Buffers	TMAC	Tm	Tm
PCR amplicon size	< 300 bp	All sizes	All sizes
Patent	No	Yes	No
Cost	+	+	++
Total time	± 3.5 hours	± 6 hours	± 7 hours

Multiplex ligation dependent amplification (LDA)



EXPERIMENTAL WORKFLOW



RESULTS

Marker selection

- AFLP fragments
- Prophage genomes
- Sequence repeats
- SGI1
- Allantoin utilization
- Antibiotic resistance
- SNPs
- Specificity *Salmonella enterica* subsp. *enterica*
- Specificity *Salmonella* Typhimurium

LDA profiles of collection STM

MLVA	Antimicrobial resistance	Phage type	Sample	SAL-1	SAL-2	SAL-11	SAL-23	SAL-29	SAL-33	SAL-49	SAL-50	SAL-51	SAL-53	SAL-55	SAL-64	SAL-67	SAL-69	SAL-70	SAL-71
3-14-11-NA-NA		DT104a	12-2721	■	■	■	■	■	-	■	-	-	-	-	-	-	-	-	-
3-17-16-11-NA	AmAmcTeCSSss	DT12	11-1015	■	■	■	■	-	-	-	■	■	■	■	-	■	■	■	■
3-15-10-NA-311	AmAmcTeNaCSss	DT104L	11-0008	■	■	■	■	-	-	-	■	■	■	■	-	■	■	■	■
3-15-10-NA-311	AmAmcTeNaCSSss	DT104L	11-0210	■	■	■	■	-	-	-	■	■	■	■	-	■	■	■	■
3-15-10-NA-311	AmAmcTeCSSss	DT104L	12-3411	■	■	■	■	-	-	-	■	■	■	■	-	■	■	■	■
3-12-10-NA-211	AmAmcTeCSSss	DT104L	11-3355	■	■	■	■	-	-	-	■	■	■	■	-	■	■	■	■
3-13-12-22-311	AmTeCSSss	DT104L	12-4072	■	■	■	■	-	-	-	■	■	■	■	-	■	■	■	■
3-13-12-22-311	AmAmcTeCSSss	DT104L	11-1970	■	■	■	■	-	-	-	■	■	■	■	-	■	■	■	■
3-13-8-NA-211	AmAmcTeCSSss	U302	11-0444	■	■	■	■	-	-	-	■	■	■	■	-	■	■	■	■
3-14-18-14-311	AmAmcSSss	DT104L	11-1160	■	■	■	■	-	-	-	■	■	-	■	-	-	■	■	■
3-14-18-14-311	AmTeCSSss	DT12	10-02975	■	■	■	■	-	-	-	■	■	-	■	-	■	■	■	■
3-12-21-14-NA	AmAmcTeCSSss	DT104L	12-1725	■	■	■	■	-	-	-	■	■	-	■	-	■	■	■	■
3-14-18-14-311	AmAmcTeCSSss	U302	10-02651	■	■	■	■	-	-	-	■	■	-	■	-	■	■	■	■
3-15-16-14-311	AmAmcCSSss	U302	12-3896	■	■	■	■	-	-	-	■	■	-	■	-	■	■	■	■
3-12-9-NA-211	AmTeSSss	DT104L	11-1129	■	■	-	-	■	-	■	-	-	-	-	-	-	-	-	-
3-14-5-18-311	NaSSss	U302	12-3443	■	■	■	■	-	-	-	■	■	■	■	-	-	-	■	■
3-14-15-22-311	TmpSSssSxt	DT104L	12-4026	■	■	■	■	-	-	-	■	■	■	■	-	-	-	■	■

LDA profile of outbreak

MLVA	Antimicrobial resistance	Phage type	Sample	SAL-1	SAL-2	SAL-11	SAL-23	SAL-29	SAL-33	SAL-49	SAL-50	SAL-51	SAL-53	SAL-55	SAL-64	SAL-67	SAL-69	SAL-70	SAL-71
3-13-10-NA-211	AmTeTmpSSssSxt	DT120	11-0079	■	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-
3-13-9-NA-211	AmTeTmpSSssSxt	DT120	12-1779	■	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-
3-13-8-NA-211		DT120	12-1123	■	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-
3-15-11-NA-211		DT193w	12-0475	■	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-
3-14-7-NA-211	AmTeTmpCSssSxt	DT195	12-1374	■	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-
3-14-7-NA-211	AmTeTmpCSSssSxt	DT195	12-1449	■	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-
3-12-10-NA-311	AmTeSSss	DT195	08-1193	■	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-
3-12-10-NA-311	AmTeSSss	DT195	08-1276	■	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-
3-12-10-NA-311	AmTeSSss	DT195	08-1278	■	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-
3-12-10-NA-311	AmTeSSss	DT195	08-1283	■	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-
3-12-10-NA-311	AmTeSSss	DT195	08-1303	■	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-
3-15-5-NA-211	AmSSss	DT120	08-1433	■	■	-	-	■	-	■	-	-	-	-	■	-	-	-	-

LDA profiles of stability experiment

Sample	Passage	SAL-1	SAL-2	SAL-11	SAL-23	SAL-29	SAL-33	SAL-49	SAL-50	SAL-51	SAL-53	SAL-55	SAL-64	SAL-67	SAL-69	SAL-70	SAL-71
11-0050	0	■	■	■	-	■	■	■	-	-	-	-	-	-	-	-	-
	50	■	■	■	-	■	■	■	-	-	-	-	-	-	-	-	-
11-0444	0	■	■	■	■	-	-	-	■	■	■	■	-	■	■	■	■
	50	■	■	■	■	-	-	-	■	■	■	■	-	■	■	■	■
11-0841	0	■	■	-	-	■	-	■	-	-	-	-	-	-	-	-	-
	50	■	■	-	-	■	-	■	-	-	-	-	-	-	-	-	-
11-3445	0	■	■	■	■	■	-	■	-	-	-	-	-	-	-	-	-
	50	■	■	■	■	■	-	■	-	-	-	-	-	-	-	-	-
12-1779	0	■	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-
	50	■	■	■	■	■	■	■	-	-	-	-	-	-	-	-	-

CONCLUSIONS AND PERSPECTIVES

Proof of concept for LDA assay for subtyping of *Salmonella* Typhimurium
→ Combination of different types of markers
→ Modular multiplex assay, currently 16-plex
→ Stability of assay confirmed *in vitro*

Perspectives:
→ Increase discriminatory power with additional markers
→ Inter-laboratory testing for harmonization

WIV-ISP | Rue Juliette Wytsmanstraat 14 | 1050 Brussels | Belgium
T +32 2 642 53 60 | F +32 2 642 52 92 | e-mail: veronique.wuyts@wiv-isp.be | website: www.wiv-isp.be